Claims

1. A method for identifying a display molecule of a bifunctional complex further comprising an identifier oligonucleotide linked to the display molecule, said method comprising the steps of

   i) separating complementary single stranded identifier oligonucleotides from an enriched and partitioned fraction of bifunctional complexes having an affinity for a target molecule, said bifunctional complexes comprising a duplex identifier oligonucleotide comprising said complementary single stranded identifier oligonucleotides,

   ii) hybridising the single stranded identifier oligonucleotides obtained in step i), thereby generating a composition comprising homo-duplex identifier oligonucleotides and hetero-duplex identifier oligonucleotides, wherein homo-duplex identifier oligonucleotides hybridise without generating any mis-matches between the complementary oligonucleotide strands, and wherein one or more mis-matches are present when complementary identifier oligonucleotides form hetero-duplexes,

   iii) separating homo-duplexes from hetero-duplexes,

   iv) obtaining a fraction comprising predominantly homo-duplexes,

   v) decoding the identifier oligonucleotides of the homo-duplexes, and

   vi) identifying based on the decoding in step v) the one or more display molecules identifier by the decoded identifier oligonucleotides.

2. The method of claim 1, wherein the duplex identifier oligonucleotide comprising complementary single stranded identifier oligonucleotides is obtained by hybridisation of a complementary identifier oligonucleotide with the identifier oligonucleotide linked to the display molecule of the bifunctional complex.

3. The method of claim 1, wherein the duplex identifier oligonucleotide comprising complementary single stranded identifier oligonucleotides is obtained by hybridising a probe oligonucleotide with the identifier oligonucleotide of the bifunctional complex, enzymatically extending said probe oligonucleotide, thereby obtaining a complementary identifier oligonucleotide hybridised with the identifier oligonucleotide linked to the display of the bifunctional complex.

4. The method of any of claims 2 and 3, wherein said duplex identifier oligonucleotide comprising complementary single stranded identifier oligonucleotides is obtained prior to, during or concomitantly with the step of partitioning the bifunctional complexes.

5. The method of any of claims 2 and 3, wherein said duplex identifier oligonucleotide comprising complementary single stranded identifier oligonucleotides is obtained after the step of partitioning the bifunctional complexes.

6. The method of claim 1, wherein the identifier oligonucleotide linked to the display molecule is selected from the group consisting of a) a single stranded identifier oligonucleotide and b) a duplex identifier oligonucleotide comprising complementary single stranded identifier oligonucleotides, said method comprising the preliminary steps of

i) providing a composition of bifunctional complexes comprising different display molecules,

ii) providing one or more target molecule(s) having an affinity for one or more of said display molecules,

iii) contacting the composition of bifunctional complexes provided in step i) with the target molecule provided in step ii)

iv) obtaining an enriched fraction of bifunctional complexes comprising one or more display molecules having an affinity for said target molecule,

v) partitioning said enriched fraction obtained in step iv) from bifunctional complexes not having an affinity for said target molecule, and

vi) complementing single stranded identifier oligonucleotides of different bifunctional complexes in which the display molecule is linked to a) a single stranded identifier oligonucleotide, thereby obtaining a duplex identifier oligonucleotide comprising complementary identifier oligonucleotides, with the proviso that no single stranded complementation occurs for bifunctional complexes comprising b) duplex identifier oligonucleotides comprising complementary identifier oligonucleotides.

7. The method of claim 6 wherein the display molecule of a bifunctional complex provided in step i) or obtained in step iv) is linked to a single stranded identifier oligonucleotide, said method comprising the further step of complementing said single stranded identifier oligonucleotides of the different bifunctional complexes, thereby obtaining a duplex identifier oligonucleotide comprising complementary identifier oligonucleotides.

8. The method of claim 7, wherein said single stranded identifier oligonucleotides of the different bifunctional complexes are complemented prior to partitioning of the bifunctional complexes.

9. The method of claim 7, wherein said single stranded identifier oligonucleotides of the different bifunctional complexes are complemented after partitioning of the bifunctional complexes.

10. The method of claim 7, wherein said single stranded identifier oligonucleotides of the different bifunctional complexes are complemented during or concomitantly with the partitioning of the bifunctional complexes.

11. The method of any of claims 7 to 10, wherein the complementation of the single stranded identifier oligonucleotides comprises the steps of hybridising one or more oligonucleotide probe(s) to the single stranded identifier oligonucleotide of at least some of the bifunctional complexes, and ligating and/or extending enzymatically said probe(s), thereby generating a duplex identifier oligonucleotide comprising complementary oligonucleotide strands.

12. The method of any of claims 7 to 10, wherein the complementation of the single stranded identifier oligonucleotides comprises the step of hybridising a complementary identifier oligonucleotide to the single stranded identifier oligonucleotide of at least some of the bifunctional complexes.

13. The method of any of claims 1 to 7, wherein the display molecule of at least some of the bifunctional complexes are linked to duplex identifier oligonucleotides comprising complementary oligonucleotide strands.

14. The method of any of claims 1 to 13, wherein hetero-duplexes are selectively removed or eliminated from the composition comprising homo-duplex identifier oligonucleotides and hetero-duplex identifier oligonucleotides.

15. The method of claim 14, wherein the hetero-duplexes are removed by enzymatically degrading the mis-matched, single stranded part of the hetero-duplexes.

16. The method of claim 15, wherein the enzyme comprises nuclease activity or consists of polypeptide having nuclease activity.

17. The method of any of claims 15 and 16, wherein the enzyme is selected from the group of enzymes consisting of T4 endonuclease VII, T4 endonuclease I, CEL I, nuclease S1, including variants and combinations thereof.

18. The method of any of claims 15 and 16, wherein the enzyme is thermostable.

19. The method of any of claims 1 to 18, wherein the display molecule is obtained by reacting two or more chemical entities, wherein each chemical entity is preferably linked to a building block comprising one or more anti-codons, wherein said anti-codon(s) is complementary to an identifier oligonucleotide codon(s), wherein said codons or anti-codons identifies the chemical entities having reacted to generate the display molecule.

20. The method of claims 1 to 19, wherein the display molecule is obtained by a method comprising the steps of

i) providing at least one template comprising a sequence of n coding elements,

wherein each coding element comprises at least one recognition group capable of recognising a predetermined complementing element, and

wherein n is an integer of more than 1,

ii) providing a plurality of building blocks, wherein each building block comprises

a) at least one complementing element comprising at least one recognition group capable of recognising a predetermined coding element,

b) at least one functional entity comprising at least one functional group and at least one reactive group, and

c) at least one linker separating the at least one functional entity from the at least one complementing element,

iii) contacting each of said coding elements with a complementing element capable of recognising said coding element,

iv) optionally obtaining a complementing template by covalently linking the complementing elements, and

v) obtaining a display molecule by reacting functional groups on different building blocks,

wherein the display molecule is linked to a building block or to the complementing template, when formed, or to the template that templated the synthesis of the display molecule.

21. The method of claims 1 to 19, wherein the display molecule is obtained by a method comprising the steps of

i) providing at least one template comprising one or more codons capable of hybridising to an anti-codon, wherein said template is optionally associated with one or more chemical entities, and
ii) providing a plurality of building blocks each comprising an anti-codon associated with one or more chemical entities,
iii) hybridising the anti-codon of one or more of the provided building blocks to the template,
iv) covalently linking said anti-codons and/or linking the at least one template with the anti-codon of at least one building block, thereby generating an identifier oligonucleotide capable of identifying chemical entities having participated in the synthesis of the display molecule,
v) displacing or separating the template from one or more of the anti-codons hybridised thereto, thereby generating an at least partly single stranded identifier oligonucleotide associated with a plurality of chemical entities,
vi) generating a bifunctional complex comprising a display molecule linked to an identifier oligonucleotide identifying the chemical entities having participated in the synthesis of the display molecule,

wherein said display molecule is generated by reacting at least two of said plurality of chemical entities associated with the identifier polynucleotide,

wherein said at least two chemical entities are provided by separate building blocks or provided by the template and at least one building block.

22. The method of any of claims 1 to 19, wherein the display molecule is obtained by a method comprising the steps of

i) providing a plurality of building blocks each comprising an oligonucleotide associated with one or more chemical entities,

ii) providing at least one connector oligonucleotide capable of hybridising with one or more building block oligonucleotides,

iii) immobilising at least one building block to a solid support,

iv) hybridising said immobilised building block oligonucleotide to a first connector oligonucleotide,

v) hybridising at least one additional building block oligonucleotide to said first connector oligonucleotide,

vi) ligating building block oligonucleotides hybridised to the connector oligonucleotide,

vii) separating the connector polynucleotide from the ligated building block oligonucleotides,

viii) reacting one or more chemical entities associated with different building block oligonucleotides, thereby obtaining a first bifunctional complex comprising a first display molecule or first display molecule precursor linked to a first identifier oligonucleotide

identifying the chemical entities having participated in the synthesis of the first display molecule or first display molecule precursor, wherein said first bifunctional complex is immobilised to a solid support.

23. The method of claim 22, wherein said chemical entities are reacted in a reaction compartment from which the connector oligonucleotide has been removed in a washing and/or separation step prior to the reaction of said chemical entities.

24. The method of claim 22 comprising the further steps of

i) providing a second connector polynucleotide,

ii) hybridising said second connector polynucleotide to the identifier polynucleotide of said first bifunctional complex,

iii) hybridising at least one further oligonucleotide of a building block to said second connector oligonucleotide,

iv) ligating building block oligonucleotides hybridised to the second connector oligonucleotide, wherein at least one of said building block oligonucleotides are hybridised to the first identifier polynucleotide,

v) separating the second connector polynucleotide from the ligated building block oligonucleotides, preferably by diverting the second connector polynucleotide to another compartment,

vi) reacting the first display molecule precursor with the one or more chemical entities associated with the ligated building block oligonucleotide(s), thereby obtaining a second bifunctional complex comprising a second display molecule or second display molecule precursor linked to a second identifier polynucleotide identifying the chemical entities having participated in the synthesis of the display molecule or display molecule precursor,

wherein said second bifunctional complex is immobilised to a solid support.

25. The method of claim 24, wherein steps i) to vi) are repeated for different connector oligonucleotides and different further building blocks.

26. The method of any of claims 1 to 19, wherein the display molecule is obtained by a method comprising the steps of

i) providing a plurality of building blocks selected from the group consisting of

a) building blocks comprising an identifier oligonucleotide linked to one or more chemical entities,

b) building blocks comprising an identifier oligonucleotide linked to one or more reactive groups, and

c) building blocks comprising an identifier oligonucleotide comprising a spacer region, wherein said building blocks comprising a spacer region are preferably connector

polynucleotides to which building blocks of groups a) and b) can hybridise,

ii) generating a hybridisation complex comprising at least n building blocks by hybridising the identifier oligonucleotide of one building block to the identifier oligonucleotide of at least one other building block,

wherein n is an integer of 4 or more

wherein at least 3 of said at least n building blocks comprise a chemical entity,

wherein no single identifier oligonucleotide is hybridised to all of the remaining identifier oligonucleotides,

wherein optionally at least one of said building blocks of group c) is immobilised to a solid support, thereby providing a handle to which an oligonucleotide of at least one building block of groups a) or b) can hybridise,

iii) covalently linking identifier oligonucleotides of building blocks comprising one or more chemical entities, thereby obtaining an identifier oligonucleotide comprising covalently linked identifier oligonucleotides each associated with one or more chemical entities,

iv) optionally separating said identifier oligonucleotide obtained in step iii) from any immobilised connector oligonucleotides hybridised thereto, wherein said separation optionally comprises the step of diverting said identifier polynucleotide

comprising covalently linked identifier oligonucleotides each associated with one or more chemical entities to a different reaction compart-ment, thereby separating said identifier polynucleotide from said immobilised connector oligonucleotides,

v) reacting said at least 3 chemical entities linked to the identifier polynucleotide, and

vi) obtaining a bifunctional complex comprising a display molecule resulting from the reaction of a plurality of chemical entities, wherein said display molecule is linked to an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the molecule.

27. The method of claim 26 wherein a plurality of bifunctional complexes comprising different display molecules is obtained by repeating the method steps for different building blocks.

28. The method of any of claims 1 to 19, wherein the display molecule is obtained by a method comprising the steps of

i) providing an oligonucleotide comprising a priming site linked to a) a reaction site comprising one or more reactive group(s) and b) a site for tag addition,

ii) providing a plurality of building blocks each comprising a probe oligonucleotide capable of hybridising with the priming site, wherein at least part of said plurality of building blocks comprise a probe oligonucleotide linked to different chemical entities,

iii) sequentially hybridising different building blocks to the priming site of the oligonucleotide provided in step i),

iv) reacting for each hybridised building block a chemical entity with a reactive group of the reaction site of the oligonucleotide provided in step i), and

v) adding for every chemical entity reacted a unique tag residue to the site for tag addition, said unique tag identifying the chemical entity having reacted with a reactive group of the reactive site.

29. The method of any of claims 1 to 19, wherein the display molecule is obtained by a method comprising the steps of

i) providing an oligonucleotide comprising a tag addition site and either
one or more reactive site for functionalization, or
a scaffold comprising one or more reactive site(s) for functionalization,

ii) reacting one or more scaffold reactive sites with two or more chemical entities, and

iii) adding for each chemical entity reacted with a scaffold reactive site a unique tag residue to the tag addition site, thereby obtaining a bifunctional complex comprising a display molecule resulting from the chemical entity reactions, said display molecule being linked to an identifier

oligonucleotide comprising the individual unique tag residues.

30. The method of any of claims 19 to 29, wherein at least 3 chemical entities, such as at least 4 chemical entities, for example at least 5 chemical entities, such as at least 6 chemical entities, for example at least 7 chemical entities, such as at least 8 chemical entities, for example at least 9 chemical entities, such as at least 10 chemical entities, for example at least 11 chemical entities, such as at least 12 chemical entities, for example at least 13 chemical entities, such as at least 14 chemical entities, are reacted.

31. The method of any of claims 19 to 29, wherein the plurality of display molecules is selected from the group consisting of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptides, polysulfonamides, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinones, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, including any combination thereof,

wherein each display molecule is synthesised by reacting a plurality of chemical entities preferably in the range of from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to

60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

32. The method of any of claims 1 to 31, wherein the chemical entities are precursor chemical entities which are processed in the synthesis of

the display molecule, wherein the processed chemical entities form part of the display molecule.

33. The method of any of claims 10 to 31, wherein each codon comprises 4 or more nucleotides.

34. The method of any of claims 1 to 33, wherein the display molecules are non-α-polypeptides.

35. The method of any of claims 1 to 33, wherein the display molecules are natural or non-natural nucleic acids.

36. The method of any of claims 1 to 33, wherein the display molecules have a molecular weight of less than 2000 Dalton, such as less than 1000 Dalton, for example less than 500 Dalton.

37. The method of any of claims 1 to 18, wherein one or more chemical entities are transferred to a display molecule or an intermediate thereof by a chemical building block further comprising an anti-codon.

38. The method of claim 37, wherein the information of the anti-codon is transferred in conjunction with the chemical entity to the bifunctional complex.

39. The method of any of claims 1 to 38, wherein the chemical entity reactions occur without the intervention of an enzyme.

40. The method of claim 19, wherein the codons are separated by a framing sequence.

41. The method of any of claims 1 to 40, wherein the linker linking the display molecule and the identifier oligonucleotide is selectively cleavable.

42. The method of claim 41, wherein the linker is cleaved by irradiation.

43. The method of any of claims 41 and 42, wherein the linker is cleaved after the bifunctional complex has contacted the target.

44. The method of claim 6, wherein the composition of bifunctional complexes comprises more than 1000 different display molecules.

45. The method of claim 1, wherein the target molecule is of a biological origin.

46. The method of claim 45, wherein the molecular target is immobilized on a solid support.

47. The method of claim 46, wherein the immobilized target forms a stable or quasi-stable dispersion.

48. The method of any of claims 1 to 47, wherein the target molecule comprises or consists of a polypeptide.

49. The method of claim 48, wherein the polypeptide is selected from the group consisting of kinases, proteases, phosphatases, and antibodies.

50. The method of any of claims 1 to 29, wherein the target molecule comprises or consists of a nucleic acid.

51. The method of any of claims 1 to 29, wherein the display molecule comprises or consists of a nucleic acid.

52. The method of any of claims 50 and 51, wherein the nucleic acid is a DNA or RNA aptamer.

53. The method of any of claims 1 to 29, wherein the target molecule, such as a polypeptide, is associated with the nucleic acid template having templated the synthesis thereof.

54. The method of any of claims 1 to 53, wherein the contacting of the target molecule and the composition of different bifunctional complexes is achieved by mixing the target molecule with the composition of different bifunctional coplexes.

55. The method of claim 54, wherein the target is saturated with a known ligand prior to contacting the different bifunctional complexes.

56. The method of any of claims 1 to 55, wherein at least one of the complementary identifier oligonucleotides of the recovered homo-duplexes is amplified prior to the step of decoding the identity of the display molecule.

57. The method of any of claims 1 to 56, wherein the partitioned fraction of identifier oligonucleotides is amplified by PCR.

58. The method of claim 57, wherein the identifier oligonucleotides are proportionally amplified.

59. The method of any of claims 1 to 58, wherein the complementary identifier oligonucleotide strands of homo-duplexes and hetero-

duplexes in the fraction comprising predominantly homo-duplexes are separated and allowed to hybridise, said hybridisation resulting in a further fraction comprising predominantly homo-duplexes.

60. The method of claim 59, wherein the complementary identifier oligonucleotide strands of homo-duplexes and hetero-duplexes in the fraction comprising predominantly homo-duplexes are amplified before being re-hybridised.

61. The method of any of claims 59 and 60, wherein the identifier oligonucleotides are decoded before being re-hybridised.

62. The method of claim 61, wherein the decoding of the identifier oligonucleotides is used to eliminate a subset of identifier oligonucleotides before the step of re-hybridisation.